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SHORT COMMUNICATION

A LYMPHOBLASTOID CELL LINE DUALLY INFECTED WITH MAREK'S DISEASE VIRUS AND AVIAN LEUKOSIS VIRUS

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One of the greatest advances in recent research on Marek's disease (MD) in vitro, is the successful establishment of lymphoid cell lines derived from MD lymphomas. The first two cell lines established from lymphomas of MD were reported by Akiyama et al. (1973) and Akiyama and Kato (1974). Details of the virological and biological characteristics of these lines were described by Kato and Akiyama (1975) and Nazerian and Witter (1975). Successful establishment of two other MD cell lines was achieved by Powell et al. (1974). The present paper reports establishment of the 5th cell line derived from MD lymphoma. Chicks were inoculated intramuscularly with 2×10^7 cells of the MSB-1 line. MD lymphomas developed in their ovaries and cultivation of single cell suspensions of the lymphomas was carried out as described previously (Akiyama and Kato, 1974). One culture derived from an ovarian tumor was found to grow after a cultivation period of about 30 days. This culture has now been growing for more than 365 days and is designated as " MOB-2 ".

The characteristics of MOB-2 line cells were mostly similar to those of the MOB-1 and MSB-1 line cells. Growth was better at 41 C than at 37 C. The cells grew singly and did not become attached to the surface of the culture vessel. Most cells were lymphoblastoid cells (Fig. 1), and they had a mean volume of 649 μ^3 measured in the living state in a Coulter counter. A comparison of their cell volume with those of other MD line cells and normal chick lymphocytes is shown in Table 1. A few percent of the cells were always MD viral antigen positive when examined by the immunofluorescent test. The percentages of viable cells in the logarithmic phase varied from 43% to 50%. MD virus (MDV) could be isolated by co-cultivation with susceptible cells, such as chick kidney cells and duck embryo fibroblasts. Most of the line cells were found to have T-surface marker by the cytotoxicity test as well as by the immunofluorescent test, as reported by Matsuda et al. (1976).

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TABLE 1. *Comparison of cell volumes*

Cells	Volume (μ^3)
MOB-2	649± 73[a]
MSB-1	665±141
MOB-1	616±112
Thymus	154± 9
Bursa	210± 11
Spleen	184± 16

[a] Standard deviation.

Chromosome analysis was kindly performed by Dr. N. Takagi, Chromosome Research Unit, Hokkaido University, by acridine orange staining (Dutrillaux et al., 1973). When cells were stained with acridine orange after 5-Bromo-2'-deoxyuridine (BUdR) incorporation for 10 hr the 3 largest chromosomes were found to have a banded appearance indistinguishable from that of the normal No. 1 (Fig. 2). Thus the MOB-2 line cells are karyologically different from those of MOB-1 and MSB-1.

The MOB-2 line cells had a similar ultrastructural appearance to other line cells. A few percent of the cells contained immature herpesvirus particles, mainly located in their nuclei. Surprisingly, abundant C particles were also found, most in extracytoplasmic spaces and some in intracytoplasmic vesicles (Fig. 3, 4). A few cells contained both herpes-

TABLE 2. *RIF test of MD cell lines on quail cells*[a]

Cell line	Relative plating efficiencies of challenge virus			
	Subgroup A		Subgroup E	
	SR-RSVA[b]	RSV (RAV[c]-1)[d]	RSV (chf)[d]	RSV (RAV[c]-60)[d]
MOB-2	0.1	0.01	1	1
MSB-1	1	1	1	1
None	1	1	1	1

[a] The RIF test was performed after co-cultivation of QEF with each MD cell line.
[b] Schmidt-Ruppin strain of Rous sarcoma virus, subgroup A.
[c] Rous-associated virus.
[d] Pseudotypes of the Bryan high titer strain.

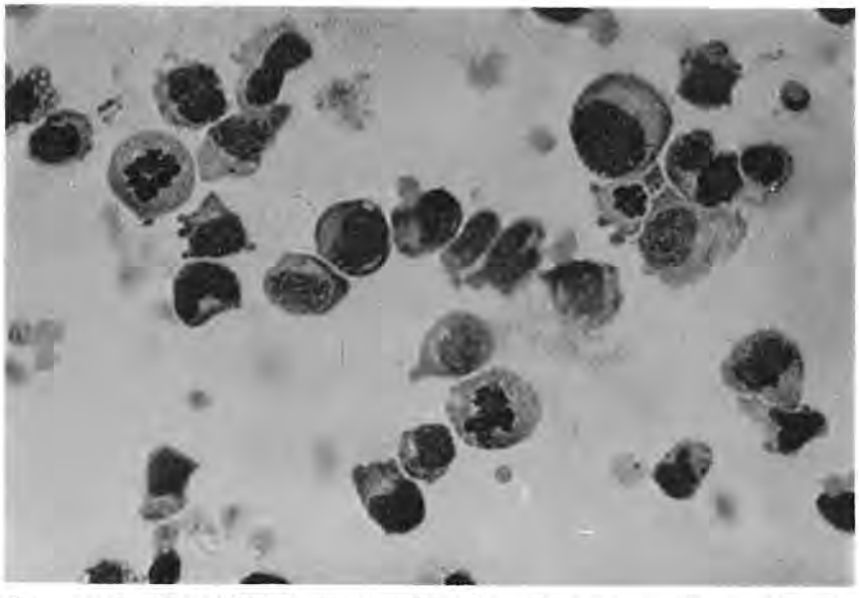

FIGURE 1. Smear preparation of MOB-2 line cells after 141 days' cultivation. Fixed with methanol, stained with Giemsa solution. The line consists of lymphoblastoid cells.

FIGURE 2. Chromosomes of a MOB-2 cell. The three largest chromosomes had a banded appearance indistinguishable from that of the normal No. 1. Stained with acridine orange after BUdR incorporation for 10 hr, and observed under an ultraviolet ray microscope.

type particles in their nuclei and C particles in their intracytoplasmic vesicles. Thus the MOB-2 cell line differs from the other cell lines, MOB-1 and MSB-1, which are free from any expression of exogenous or endogenous avian leukosis virus (ALV) (Ikuta et al., 1976).

The complement-fixation for ALV and gs antigen tests were both positive, while the immunofluorescent test for avian reticuloendotheliosis virus was negative. The resistance-inducing factor (RIF) test was carried out to determine whether these C particles came from exogenous or endogenous ALV. Quail embryo fibroblasts (QEF) were co-cultivated with 1×10^6 line cells and transferred 3 times at 3 or 4 days intervals. The final cultures were challenged with avian sarcoma viruses of subgroups A and E. As shown in Table 2, the C particles found in MOB-2 line cells were exogenous ALV, subgroup A. No markers of transformation by ALV have been reported, so it is unknown whether MOB-2 line cells are double transformant or not. Osato and Nonoyama (1974) obtained a cell line, named FVNC, by infection of Epstein-Barr virus (EBV)-nonproductive human lymphoid NC-37 cells with Friend murine leukemia virus (FLV). The FVNC cell line has been maintained free of detectable EBV- and FLV-related immunofluorescent antigens, but both viral genomes exist in the cells in a repressed form. This paper reports the first example of MD lymphoma line cells dually infected with ALV. In contrast to FVNC, this line named the MOB-2 line, maintains both MDV and ALV in an expressed form. We have not determined whether the exogenous ALV came from the MD chick. Ikuta et al. (1976) showed that the MOB-1 and MSB-1 lines established previously, lack any expressions of endogenous [gs, chick helper factor (chf)] or exogenous avian RNA tumor viruses. This may indicate that the exogenous ALV at least is not necessary for establishment of the cell lines. Further studies are required on the effect of superinfection of exogenous ALV on the characteristics of MD lymphoma line cells.

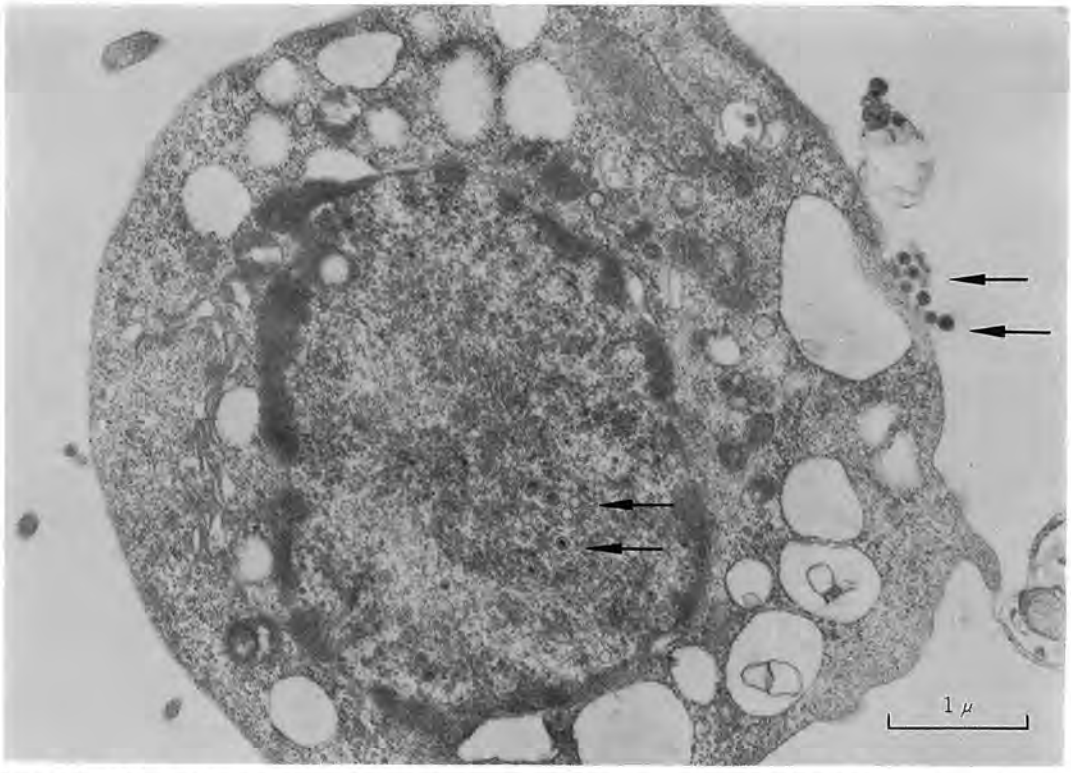

FIGURE 3. Thin section electron micrograph of a lymphoblastoid cell of the MOB-2 line. Several herpes-type capsid structures in the nucleus and several C particles in the extracellular space are observed (arrows).

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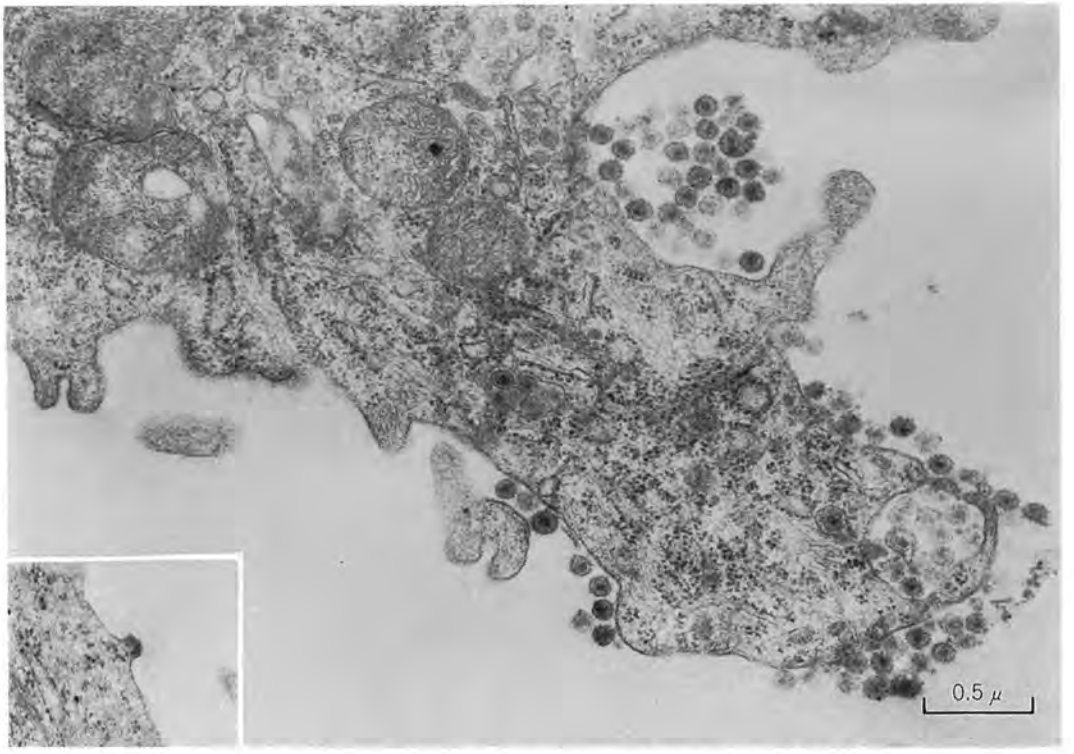

FIGURE 4. Thin section electron micrographs of cells of the MOB-2 line. Typical C particles in the extracellular space and a particle budding at the cell surface (insert) are seen.

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